increased dissociation rates, if desired. An optimized variable region exhibiting lower affinity binding is useful, for example, for penetrating a solid tumor. In contrast to a higher affinity variable region, which would bind to the peripheral regions of the tumor but would be unable to penetrate to the inner regions of the tumor due to its high affinity, a lower affinity variable region would be advantageous for penetrating the inner regions of the tumor. As with optimization of binding affinities above, optimization of a catalytic variable region can be, for example, increased or decreased catalytic rates, disassociation constants or association constants.

As used herein, the term "substantially the same" when used in reference to binding affinity is intended to mean similar or identical binding affinities where one molecule has a binding affinity constant that is similar to another molecule within the experimental variability of the affinity measurement. The experimental variability of the binding affinity measurement is dependent upon the specific assay used and is known to those skilled in the art.

The invention provides a method for conferring donor CDR binding affinity onto an antibody acceptor variable region framework. The method consists of: (a) constructing a population of altered antibody variable region encoding nucleic acids, the population consisting of encoding nucleic acids for an acceptor variable region framework containing a plurality of different amino acids at one or more acceptor framework region amino acid positions and donor CDRs containing a plurality of different amino acids at one or more donor CDR amino acid positions; (b) expressing the population of altered

variable region encoding nucleic acids, and (c) identifying one or more altered variable regions having binding affinity substantially the same or greater than the donor CDR variable region.

The process of producing human antibody forms from nonhuman species involves recombinantly splicing CDRs from a nonhuman donor antibody into a human acceptor framework region to confer binding activity onto the resultant grafted antibody, or variable region binding fragment thereof. The process of grafting, referred to as the procedure for splicing CDRs into a framework, while mechanically simple it almost always results in a grafted antibody that exhibits a substantial loss in binding affinity. Although donor and acceptor variable regions are structurally similar, the process nevertheless combines CDR binding domains with a heterologous acceptor region, resulting in a conformationally imperfect setting for the binding residues of the grafted antibody. Therefore, once the CDR-grafted antibody, or variable region binding fragment is made, it requires subsequent rounds of molecular engineering to reacquire binding affinity comparable to the donor antibody. The present invention combines these steps such that CDR grafting and binding reacquisition occur in a single simultaneous procedure. The method is also applicable to optimizing the binding affinity of an antibody, or variable region binding fragment simultaneous with CDR grafting and to optimizing an antibody or variable region binding fragment in a single procedure without including the CDR grafting process.

The methods of the invention confer or impart donor CDR binding affinity onto an antibody acceptor variable region framework in a procedure which achieves

grafting of donor CDRs and affinity reacquisition in a simultaneous process. The methods similarly can be used, either alone or in combination with CDR grafting, to modify or optimize the binding affinity of a variable region. The methods for conferring donor CDR binding affinity onto an acceptor variable region are applicable to both heavy and light chain variable regions and as such can be used to simultaneous graft and optimize the binding affinity of an antibody variable region.

The methods for conferring donor CDR binding affinity onto a variable region involve identifying the relevant amino acid positions in the acceptor framework that are known or predicted to influence a CDR conformation, or that are known or predicted to influence the spacial context of amino acid side chains within the CDR that participate in binding, and then generate a population of altered variable region species that incorporate a plurality of different amino acid residues at those positions. For example, the different amino acid residues at those positions can be incorporated either randomly or with a predetermined bias and can include all of the twenty naturally occurring amino acid residues at each of the relevant positions. Subsets, including less than all of the naturally occurring amino acids can additionally be chosen for incorporation at the relevant framework positions. Including a plurality of different amino acid residues at each of the relevant framework positions ensures that there will be at least one species within the population that will have framework changes which allows the CDRs to reacquire their donor binding affinity in the context of the acceptor framework variable region.